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ABSTRACT: Radiocarbon dating, with special reference to the modern bomb-curve, can provide useful information to elucidate the date of death of skeletonized human remains. Interpretation can be enhanced with analysis of different types of tissues within a single skeleton because of the known variability of formation times and remodeling rates. Analysis of radiocarbon content of teeth, especially the enamel in tooth crowns provides information about the date of formation in the childhood years and in consideration of the known timing of tooth formation can be used to estimate the birth date after 1950 A.D.

Radiocarbon analysis of modern cortical and trabecular bone samples from the same skeleton may allow proper placement on the pre-1963 or post-1963 sides of the bomb-curve since most trabecular bone generally undergoes more rapid remodeling than does most cortical bone. Pre-1963 bone formation would produce higher radiocarbon values for most trabecular bone than for most cortical bone. This relationship is reversed for formation after 1963.

Radiocarbon analysis was conducted in this study on dental, cortical and trabecular bone samples from two adult individuals of known birth (1925 and 1926) and death dates (1995 and 1959). As expected, the dental results correspond to pre-bomb-curve values reflecting conditions during the childhoods of the individuals. The radiocarbon content of most bone samples reflected the higher modern bomb-curve values. Within the bone sample analyses, the values of the trabecular bone were higher than those of cortical bone and supported the known placement on the pre-1963 side of the bomb-curve.

KEYWORDS; forensic science, radiocarbon, skeletons, date of death, bomb-curve

The estimation of time since death represents an important but frequently elusive goal in the forensic analysis of human skeletal remains. When skeletonized human remains are found in medico-legal contexts, it is usually desirable to determine the post-mortem interval as accurately as possible. Although observations on odor and the extent of preservation can provide useful information, research and casework have demonstrated the inherent difficulty in estimating time since death precisely from morphology alone. Many variables can affect tissue preservation, and usually at least some of these variables are unknown in forensic cases. In some skeletonized cases, it is not possible to use morphological indicators to distinguish remains dating back decades or even centuries from those of relatively recent individuals who are of forensic interest. In such cases, detection of anthropogenic radiocarbon may provide additional clues.

Between 1950 and 1963, atmospheric testing of thermonuclear devices produced artificially elevated levels of carbon-14 (^{14}C)in terrestrial organisms, including humans. Levels of ^{14}C increased dramatically after 1950 to a peak in about 1963. Following a subsequent reduction and cessation of such testing, levels have declined steadily since 1963 but still remain above the pre-1950 level (figure 1). As noted by Taylor et al. 1989; Ubelaker, 2001; Ubelaker and Houck, 2002; and Wild et al. 2000, analysis of radiocarbon content and thoughtful comparison with bomb-curve values can distinguish human remains from individuals who died prior to 1950 from those who were alive after that date.

Interpretation of radiocarbon data and time since death issues is complicated by the fact that the post 1950 curve displays an increase between 1950 and 1963 and a post-

1963 decline, thus offering two possible dates for the plotting and interpretation of most radiocarbon values. If radiocarbon analysis produces a single elevated value, it can be difficult to place on the post-1950 curve unless it happens to fall at the apex of the curve in 1963 or other temporal information is available.

An additional complication relates to the complexity of bone formation and turnover dynamics. The radiocarbon value derived from analysis of human tissue reflects environmental conditions at the time the tissue formed and to some extent the diet of the individual and other features. In all cases the tissue radiocarbon level is an average of the radiocarbon values of the source carbon used to construct those tissues. In newborns and children whose bones are growing rapidly, radiocarbon values of most tissues likely are closely linked to the date of death. However, in adults, especially the elderly, radiocarbon values of cortical bone collagen are long term averages of bone formation and remodeling rather than the precise moment of death The relatively few data published on radiocarbon values derived from bone samples of individuals of known birth and death dates, suggest that in elderly individuals the average bone formation dates indicated by the radiocarbon values may be many years earlier than the death dates, apparently due to slow bone turnover in those individuals (Harkness and Walton, 1972; Stenhouse and Baxter, 1979; Wild et al. 2000; Shin et al. 2004).

Variation within tissues

The published literature documents considerable variation within human tissues regarding concentrations of bomb-curve radiocarbon. Such variation reflects differences among various tissues in their turnover/replacement rates. Broecker et al. (1959) noted a lag time of concentrations in adult tissue vs. the atmosphere of 1.1 years for blood, 1.8

years for lung and much greater time periods for collagen within cartilage. Nydal et al. (1971) found close agreement between values from hair and blood in humans. Harkness and Walton (1972), Stenhouse and Baxter (1979), and Wild et al. (2000) all suggest that collagen in bone and cartilage and bone mineral provide delayed values of bomb-radiocarbon due to their slower turnover compared to that of hair, blood or soft tissues of the body. These studies suggest that if hair, nails, blood or soft tissues are sufficiently preserved, they would be the materials of choice for radiocarbon analysis since the radiocarbon values reflecting their time of formation would be closer to the date of death then would bone collagen or apatite. Although even with these tissues, values within the bomb-curve period would not reveal if they fell on the pre-1963 or post-1963 side of the curve.

Bone analysis

In the absence of blood, hair or soft tissue (skeletonized cases) investigators must resort to analysis of hard tissue (Ubelaker and Houck, 2002). Such analysis may reveal if remains are more modern than 1950 but interpretation of individual bone analyses must consider not only plots on the bomb curve but also the potential difference between bone formation date (documented in the radiocarbon analysis) and the death date, which may be considerable, especially in older adults.

Potential to resolve this issue rests with the understanding that different hard tissues of the body form and remodel at different ages and rates. For example, dental enamel forms during the childhood years at different ages depending on the particular tooth and does not remodel during the lifetime of the individual. Thus, radiocarbon analysis of dental enamel would capture the value and thus the date during the childhood

years at the time of formation of the particular tooth crown. The difference in age of formation of different teeth (e.g., incisor vs. molar) can provide distinct dates separated by several years (Nolla, 1960, Bolanos et al. 2000).

Bone also forms during the childhood years but continues to remodel throughout the lifetime of the individual. Evidence also suggests that bone turnover of most cortical bone such as that found in the midshaft area of long bones would be much slower than in the more porous trabecular bone, such as that found in the ends of the long bones, centra of vertebrae etc. (Enlow, 1963, Manolagas and Jilka, 1995). Radiocarbon analyses of these different tissues from the same individual should provide three distinct radiocarbon values, three distinct dates of possible tissue formation and an opportunity to plot all of the values on the bomb-curve and thus assist in elucidating the likely age at death of the individual. Theoretically, the value of the more rapidly remodeling trabecular bone would be closer to the age at death of the individual than the value of carefully selected cortical bone in the same individual. Thus if the bones were formed between 1950 and 1963, the trabecular value should be slightly higher than that of cortical bone. If the bones were forming after 1963, this relationship should be reversed; namely the value of the trabecular bone would be less than that of the cortical bone. Of course, if the individual was born shortly before or after 1950, the enamel value would reveal the age of enamel formation and this would fall on the bomb curve earlier than both the cortical bone and cancellous bone values. Such an enamel value would be useful to establish the birth date after consideration of the likely age of crown formation of the particular tooth analyzed. Depending on the age of the individual, the relationship of the death date to the bomb-curve and the success of obtaining radiocarbon values from these three tissues, the

approach should provide enhanced interpretation of the date of death in a skeletonized forensic skeleton.

Materials and Methods.

Samples were extracted for radiocarbon analysis from two adult human skeletons in the collections of the Department of Anthropology of the National Museum of Natural History of the Smithsonian Institution in Washington, D.C. Specimen 387662 represents a White female born in 1925 who died in 1995 at the age of 70 years. Samples were taken for analysis from the anterior midshaft of the right femur (cortical bone), the body of a lumbar vertebra (cancellous bone) and the crown of a left mandibular canine.

Specimen 1566 represents a White female born in 1926 who died in 1959 at the age of 33 years. Samples were collected for analysis from the anterior lower midshaft area of the right femur (cortical bone), the body of a thoracic vertebra (cancellous bone) and the mandibular left lateral incisor and mandibular left canine.

Note that exterior bone surfaces were ground away before sampling. All contact equipment was replaced (sanding disc and cutting disc) and machinery was cleaned thoroughly between samplings. Samples were shipped to the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory for analysis with information provided about the specimen number, type of bone and tooth but other information about the two individuals (age at death, date of death etc.) was withheld.

Cortical and trabecular bone specimens were further sub-sampled to eliminate any transitions between bone types and obtain the appropriate size for collagen extraction.

These sub-samples were cut into 5-10 pieces with a high speed hand held saw. The tooth root specimen 1566 was cut from the crown of the tooth and then treated as the bone samples. The dentin sample from specimen 387662 was cut from the center of the tooth that was split laterally. The separated dentin was treated as bone. Bone was decalcified by placing in 0.25 N HCl (trabecular) or 0.5 N HCl (cortical, dentin, root) at 4°C for 24 h. After a visual inspection to confirm that decalcification was complete, all samples were rinsed 3 times with distilled water and then placed in 0.01 N HCl at 60°C for 16 h to unfurl the collagen and then rinsed 3 times with DI water. Samples were transferred to quartz combustion tubes and dried on a heating block prior to addition of excess CuO, evacuation, and sealing with a H_2/O_2 torch. Samples were placed in a 900°C oven for 3.5 h and combusted to CO_2. The quartz combustion tube was cracked in an evacuated gas rig and the CO_2 was purified, trapped, and reduced to graphite in the presence of iron catalyst in individual reactors (Vogel et al, 1987).

Enamel subsamples were also removed from the crowns with a high speed hand held saw. Enamel chips were inspected to ensure that they were free of dentin. If any dentin was detected, it was removed with a low speed mill. Enamel is essentially a mineral with very low carbon content (~ 0.5% C). Enamel chips were hydrolyzed to CO_2 in individual reaction chambers, evacuated, heated and acidified with orthophosphoric acid at 90°C. The evolved CO_2 was purified, trapped, and reduced to graphite as above (Vogel et al., 1987). Graphite targets were measured at the Center for Accelerator Mass Spectrometry at Lawrence Livermore National Laboratory (LLNL).

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Results

Results of the radiocarbon analyses of the eight samples from the two individuals are presented in table 1. The $\delta^{13}C$ values for collagen (-21±2) and enamel (-15±2) are estimates based on historical experience at LLNL for subjects possessing a normal northern hemisphere diet. Radiocarbon concentrations are expressed in $F^{14}C$ and $\Delta^{14}C$ nomenclatures. The $\Delta^{14}C$ convention (Stuiver and Polach, 1977) is the standard established for reporting radiocarbon data in chronological and geophysical studies decay corrected to 1950, but was not designed for reporting post bomb data.. The $F^{14}C$ convention (Reimer et al, 2004) is designed for reporting bomb-curve data with a $\delta^{13}C$ fractionation correction and is better suited to this application. $\Delta^{14}C$ was calculated using the following formula:

$$\Delta^{14}C = 1000 * \{F^{14}C * \exp[\lambda*(1950 - y)] - 1\}$$

$F^{14}C$ is defined in Eq. 2 of Reimer et al (2004). It is enrichment or depletion of ^{14}C relative to oxalic acid standard normalized for isotope fractionation ($\lambda = 1/8267\ yr^{-1}$, y = year of measurement after 1950 A.D.). Corrections for background contamination introduced during AMS sample preparation were made following the procedures of Brown and Southon (1997).

As expected, all dental structures possessed $F^{14}C < 1.0$ and thus predate the bomb-curve. Since the two individuals examined were born in 1925 and 1926 respectively, their mandibular incisor and canine crowns would have formed between the ages of six months and four years and thus between the years 1925 and 1930, decades before the bomb-curve began. The tooth root of 1566 would have formed between ages four and seven or between the years 1930 and 1933, also long before the commencement of the bomb-curve.

Radiocarbon values for all of the bone samples but one contained bomb carbon. The exception was a cortical bone sample of specimen 1566 (lab analysis number 110255) which like the dental samples produced a $F^{14}C$ <1.0, indicating that the bone collagen in that analysis contained less than 1% post 1954 carbon although death occurred in 1959. This is significant in bone/bomb-curve interpretation since it suggests at least a five year absence in new carbon incorporation in cortical bone formation an individual who died at the relatively early age of 33.

As expected, analysis of the trabecular bone sample from the individual 1566 (lab analysis 110113) produced a fraction value of 1.031, well within the bomb-curve. Since this individual died in 1959 prior to the bomb-curve reaching its peak in 1963, the fraction value of 1.031 can be compared with the bomb-curve values prior to 1963. Such a comparison suggests an average date of formation of the trabecular bone used in the analysis of approximately 1954, or only five years prior to death.

Although individual 387662 was born in 1925, one year earlier than individual 1566, she lived until age 70 with a death date of 1995. As expected, radiocarbon analysis of both the cortical and trabecular bone samples from this individual revealed $F^{14}C$ values on the bomb curve. The values of both the cortical (1.131) and the trabecular (1.140) are slightly below the possible values for the post-1963 aspect of the bomb-curve and thus reflect average formation between 1950 and 1963. Comparison of these values with the bomb-curve suggests formation dates of approximately 1956 for the cortical bone and 1957 for the trabecular bone. As with the analysis of specimen 1566, these values suggest more recent formation dates for the trabecular bone in comparison with the

cortical bone, although the difference in this individual was only one year. The two bone values predate death by 38 and 39 years.

Discussion

The results presented above demonstrate that radiocarbon analysis of hard tissues can be useful in clarifying issues regarding date of death, but special consideration must be given to the type of tissue examined and the age of the individual. As expected, values derived from teeth, cortical bone and trabecular bone from the same individuals reveal different and sequential formation dates for the respective tissues. The early forming teeth provide the earliest values, followed by the cortical bone and then the trabecular bone. While this sequence was produced in the evaluation of tissues from both individuals, the separation among them varied considerably, reflecting the much greater age at death of individual 387662. Although diet, disease, treatment of disease and other factors can influence bone formation and remodeling rates, age seems to be a very important attribute, worthy of consideration in using this approach to estimate the approximate date of death.

Of particular importance in this study is the predictable relationship of values derived from dental samples, cortical bone and trabecular bone. In forensic analysis of an unknown skeleton, values from these three tissues provide information of the dates and sequence of bone formation and enhance the interpretive aspect of placement of the values on the bomb-curve. In particular, the relationship of the values of the cortical bone and the trabecular bone assists in the determination of whether the values fall prior to 1963 or after it. If bone formation occurred during the steep incline of the curve prior to 1963, the value of the trabecular bone should be greater than that of the cortical bone,

as documented in both individuals in the research reported here. Conversely, if tissue formation occurred, after 1963, these values should be reversed, namely the value of trabecular bone should be less than that of the cortical bone. Note also that turnover rates can vary within different types of both cancellous and cortical bone (Parfitt, 2002). Such regional locations of subperiosteal, intracortical, and subendocortical show turnover variation. Differences also should be expected between cancellous bone located in areas associated with yellow or fatty marrow vs. those associated with red or hematopoietic marrow (Parfitt, 2002).

If modern radiocarbon values with fractions above 1.0 are found in dental samples, then they can be used directly to calculate date of birth or even the death date in immature individuals, allowing for the timing of dental formation of the particular tooth type used in the analysis (Nolla, 1960; Moorrees, Fanning and Hunt, 1963a,b; Ubelaker, 1999; Bolanos et al, 2000). The slow turnover observed in collagen carbon appears to contradict the accepted rates of trabecular and cortical bone turnover of 25% and 3% annually (Manolagas and Jilka, 1995). Recent work using flooding doses of stable isotope labeled proline, a principle amino acid of collagen, indicated average collagen synthesis rates in the human iliac crest bone near 1.5%/day (Babraj et al, 2005). It is clear that the carbon in collagen is not replaced at these rates, even if the collagen is remodeled frequently. A probable explanation is recycling of the amino acids in collagen. Jackson and Heininger (1975) measured recycling of isotope labeled proline in the rat skin collagen at 93%. The extent of amino acid recycling in human collagen is not documented, but our indicates that the vast majority of the collagen is recycled. Still, we clearly see newer carbon in the collagen of trabecular bone. Placing a precise date of

death on skeletized remains based on the radiocarbon content is not yet possible. The data presented here and elsewhere in the literature strongly suggest that age at death should be considered in the interpretation of radiocarbon data. In the analysis of immature remains, both dental and skeletal samples should provide information closely linked to the actual date of death and selective analysis of different tissues should allow proper placement on the bomb-curve. With adults, especially those with advancing age, consideration should be given to the apparent reduced bone turnover and the considerable gap that may exist between bone formation and the death date.

Conclusions

Radiocarbon analysis, with special attention to placement of values on the modern bomb-curve can provide useful information in the interpretation of the date of death. Precision of interpretation is enhanced with consideration of the age of the individual and analysis of different tissues with distinct formation timing within the individual. Values obtained from different tissues within a single individual can enhance proper placement on the bomb-curve. Consideration must be given to the age at death of the individual and recognition that the dynamics of bone formation is affected by age, diet, disease, treatment of disease and other factors.

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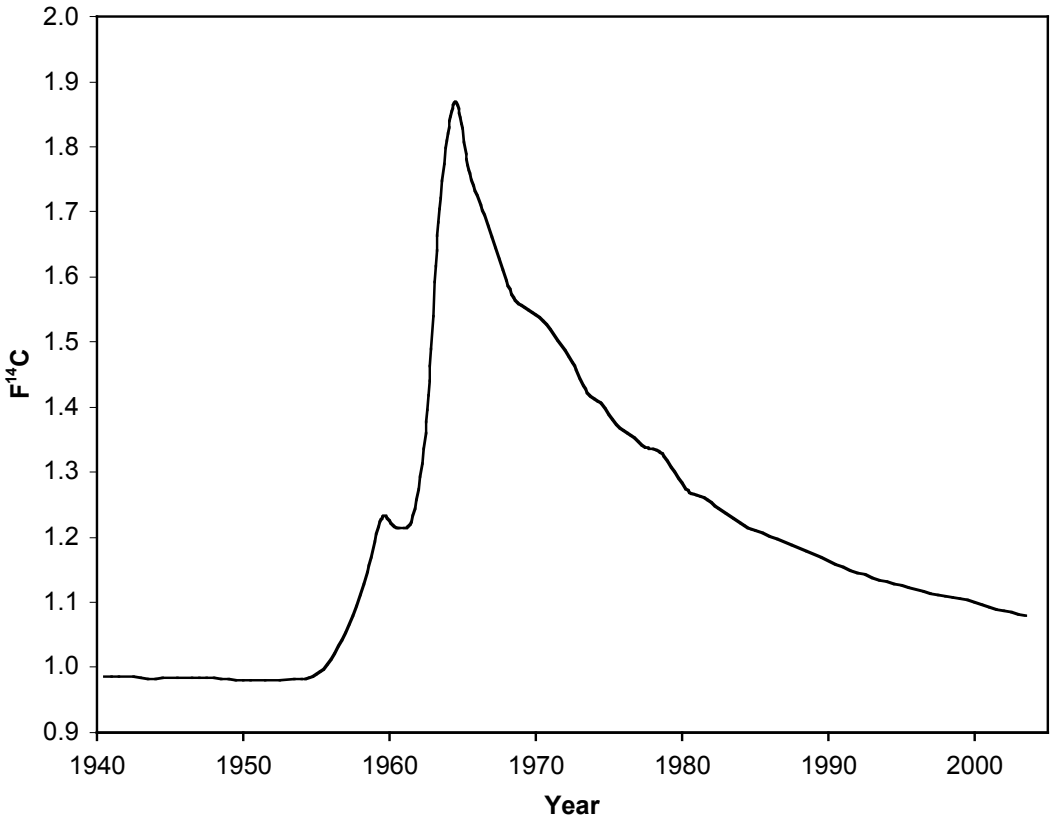

Figure 1. Average annual atmospheric $^{14}CO_2$ record for the Northern Hemisphere. Annual averaging smoothes the curve and reduces the 1963 peak. Adapted from Levin and Kromer (2004) and Stuiver et al, (1998).

Labotatory No.	Sample Name	$\delta^{13}C$	$F^{14}C$	±	$\Delta^{14}C$	±	Sample Mass (mg)	Type of Tissue	Material Analyzed
110113	1566-tb	-21 ± 2	1.031	0.005	24.8	5.4	118	trabecular	collagen
110114	1566-root	-21 ± 2	0.973	0.004	-32.9	4.3	131	tooth root	collagen
110115	1566-eml	-15 ± 2	0.977	0.004	-29.6	4.4	58.0	enamal	mineral
110116	387662-ct	-21 ± 2	1.131	0.005	123.8	5.1	114	cortical	collagen
110117	387662-tb	-21 ± 2	1.140	0.005	133.0	5.1	127	trabecular	collagen
110118	387662-eml	-15 ± 2	0.991	0.004	-15.4	4.5	53.3	enamel	mineral
110255	1566-ct	-21 ± 2	0.979	0.004	-27.0	3.6	116	cortical bone	collagen
110256	387662-den	-21 ± 2	0.992	0.005	-14.6	5.4	62.4	dentin	collagen